Conformational Dynamics and Responsiveness of Weak and Strong Polyelectrolyte Brushes: Atomistic Simulations of PDMAEMA and PMETAC

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ABSTRACT

The complex solution behavior of polymer brushes is key to control their properties, including for biomedical applications and catalysis. The swelling behavior of poly(dimethyl aminoethyl methacrylate) (PDMAEMA) and poly(2-(methacryloyloxy)ethyl trimethylammonium chloride) (PMETAC) in response to changes in pH, solvent and salt types has been investigated using atomistic molecular dynamics simulations. PDMAEMA and PMETAC have been selected as canonical models for weak and strong polyelectrolytes whose complex conformational behavior is particularly challenging for the development and validation of atomistic models. The GROMOS-derived atomic parameters reproduce the experimental swelling coefficients obtained from ellipsometry measurements for brushes of 5 to 15 nm thickness. The present atomistic models capture the protonated morphology of PDAMEMA, the swollen and collapsed conformations of PDMAEMA and PMETAC in good and bad solvents and the salt-selective response of PMETAC. The modular nature of the molecular models allows for the simple extension of atomic parameters to a variety of polymers or copolymers.

INTRODUCTION

Polymer brushes display unique physico-chemical properties that make them particularly attractive for applications in the biomedical field as anti-fouling and anti-bacterial coatings, for the design of biosensors, for cell culture and gene delivery. The ease with which the chemistry of polymer brushes can be tuned, almost independently from the chemistry of the underlying substrate, has enabled the simple design of surface physico-chemical properties.{Barbey, 2009, Polymer Brushes via Surface-Initiated Controlled Radical Polymerization: Synthesis`, Characterization`, Properties`, and Applications;Krishnamoorthy, 2014, Surface-initiated polymer brushes in the biomedical field: applications in membrane science`, biosensing`, cell culture`, regenerative medicine and antibacterial coatings} The exquisite physico-chemical behavior of polymer brushes relies on two main characteristics. On one hand, the dense packing of polymers in a brush forces their chains to stretch away from the interface due to steric hindrance and/or electrostatic repulsion. On the other hand, defined chemical functions such as ionic groups that can dissociate into polyvalent ions and counterions of opposite charge in water, regulate the conformational behavior of polymer brushes in response to variations in pH, ionic strength, temperature, solvent selectivity and salt type. Changes in these physico-chemical parameters can either screen or induce interchain interactions, triggering either the collapse or expansion of polymer chains in the brush.

Although there is a general appreciation of the importance of stimuli-responsive brushes for several biomedical applications, a detailed understanding of the molecular interactions underlying such behavior remains incomplete. For instance, polymer brushes based on oligo(ethylene glycol) methacrylate (POEGMA), sulfobetaine methacrylate (PSBMA),

carboxybetaine acrylamide (PCBAAm) and hydroxypropyl methacrylamide (PHPMAm) display exceptional protein resistance properties, but will exhibit fouling when exposed to complex biological samples such as blood or serum.{Krishnamoorthy, 2014 #693;Rodriguez Emmenegger, 2009 #703;Rodriguez-Emmenegger, 2011 #702} In particular, proteins such as apolipoprotein can adhere to POEGMA and PSBMA brushes but not PCBAAm or PHPMAm (as these coatings do not display significant fouling in serum or plasma).{Gunkel, 2013 #704;Riedel, 2013 #705} The protein resistance of polymer brushes such as POEGMA and PCBAAm has made these coatings attractive to prevent non-specific adsorption and enabled the improvement of biosensor detection, or the stability of cell micro and nanopatterns for cell-based assays.{Gautrot, 2010 #742;Gautrot, 2012 #755;Riedel, 2014 #825;Tan, 2011 #822;Vaisocherová, 2014 #824} Furthermore, these protein resistant brushes, as well as highly negatively charged SBMA, have been used to prevent the adsorption of bacteria to surfaces.{Rzhepishevska, 2013 #826} Recently, the high grafting density of positively charged polymer brushes has been shown to enable the high stability binding of oligonucleotides, making these brushes particularly promising for gene and small RNAs (siRNA and miRNA) delivery.{Krishnamoorthy, 2017 #700;Li, 2018 #746} In particular, cationic polymer brushes such as poly(dimethyl aminoethyl methacrylate) (PDMAEMA) has shown the potential to capture oligonucleotides, in particular small RNAs relevant for siRNA and miRNA delivery.{Li, 2018 #746} It was further observed that RNA interactions, specifically with oligonucleotides ranging in size between 20-30 base pairs, are more stable than for corresponding DNA molecules of similar sizes. However, it is unclear how molecular interactions shape these processes

Several experimental platforms such as surface plasmon resonance, quartz crystal microbalance (QCM), *in situ* ellipsometry and neutron reflectivity have been used to investigate brush-biomolecule interactions and brush conformation.{Krishnamoorthy, 2014 #693;Wei, 2014 #756}

However, it remains relatively challenging to probe the complex conformational behavior underlying these macroscopic observations via direct experimental measurements. Most experimental techniques can only provide limited atom-level information on the conformational behavior of brushes and their interactions with the surroundings.{Das, 2015 #737} In this context, molecular dynamics (MD) simulations are a particularly attractive method to explore the conformational response of polyelectrolytes to environmental stimuli. Yet, its reliability depends on the adequate choice of essential degrees of freedom (*i.e.* electronic, nuclear, atomic and molecular) and of interaction potentials (*i.e.* quantum or classical mechanics) governing the motion along these degrees of freedom.{Riniker, 2012 #776} Different levels of coarse-grained representations have been successfully combined with MD simulations to investigate the structural and thermodynamic properties of conventional polymer brushes.{Dimitrov, 2007 #740;Rossi, 2012 #781;Singh, 2015 #753;Honghong, 2018 #782;Wang, 2017 #783;Menichetti, 2017 #844;Steve, 2004 #846;Spyriouni, 2007 #845;Xia, 2017 #847} However, fewer systematic studies have reported on atomistic MD simulations of polymer brushes.{Benková, 2011 #832;Rodríguez-Ropero, 2013 #831;Dahal, 2017 #775} These reports have demonstrated the requirement of an atomic description to properly account for the role of solvent on polymer chain dynamics and interactions, and ion specific effects on the structure, mechanics and interfacial softness of polymer brushes.{Benková, 2011 #832;Rodríguez-Ropero, 2013 #831;Dahal, 2017 #775;Vogler, 1998 #773;Murakami, 2016 #778;Stanzione, 2015 #868} Moreover, reliable coarse-grained models are expected to reproduce molecular properties of the underlying atomistic system so that accurate atomistic models are also necessary for the development of robust CG models.{Wagner, 2016 #830} Furthermore, the properties of strong and weak polyelectrolyte brushes have been extensively investigated via continuum models based on the scaling{de Gennes, 1980

#649;Milner, 1989 #762} and self-consistent field{Zhulina, 1997 #870;Zhulina, 1991 #871;Zhulina, 1991 #869} theories. These methods have provided fundamental insights into the complex behavior of polyelectrolyte brushes under different pH regime and concentration of monovalent and multivalent salt.{Borisov, 1994 #873;Zhulina, 1995 #874;Zhulina, 2011 #872} However, the implicit representation of chemical structure (*i.e.* non-atomic description) underlying these models can only provide a limited account of the molecular dynamics and interactions of polymer chains, solvent molecules and salt. On the other hand, the complex conformational behavior of polyelectrolyte brushes in response to different solvents, pH and salts is particularly well suited for the development and validation of atomistic models, which can offer a complementary perspective to continuum models.

It is well established that charged polyelectrolyte brushes exhibit strong swelling in aqueous solutions as a result of hydration and electrostatic repulsion between brush repeat units, and collapse in apolar solvents with charged groups screened by ions from the aqueous solution.{Barbey, 2009 #735} In the case of weak polyelectrolytes such as PDMAEMA and PDEA, the hydration strongly depends on the pH and protonation states of repeat units.{Sanjuan, 2007 #770;Willott, 2014 #818} This responsive behavior is also associated with a moderate sensitivity to the ionic strength at low pH, as positive charges in the side chains of repeat units are screened by free electrolytes, as evidenced by QCM-D and ellipsometry data.{Zhang, 2017 #758} In comparison, strong polyelectrolytes do not typically respond to variations in pH but present complex conformational changes in response to variations in electrolyte type and ionic strength. For example, polycationic PMETAC brushes display different swelling behavior in presence of chaotropic anions, such as perchlorate ions, in comparison to more kosmotropic ions such as chlorides.{Alonso-García, 2013 #771} It was also shown that PMETAC brushes containing 67%

water lost 17% water during collapse in 0.1 M NaCl, whereas the collapse in 0.1 M $NaClO_4$ led to a loss of 54% of entrapped water.{Alonso-García, 2013 #771} Similar observations were reported for other strong polycationic brushes such as poly (vinylbenzyltrimethylammonium chloride) (PVBTMAC).{Bibi, 2012 #772} QCM-D data gave evidence for dramatic changes in brush hydration upon exposure to chaotropic ions. Indeed strong counter ion condensation between the weakly hydrated trimethyl ammonium group and the weakly hydrated chaotropic ions such as SCN^-, ~~ClO_3~~ClO_4^- and NO_3^- ions resulted in the rapid collapse of the polymer brush. In contrast, weak ion pairing with kosmotropic anions such as $H_2PO_4^-$, F^-, HCO_3^- and Cl^- was observed. Similarly, Kou *et al.* studied the same Hofmeister anion series using PMETAC brushes and confirmed these observations.{Kou, 2015 #819} Indeed, they evidenced a stronger concentration-dependence of brush collapse in the presence of chaotropic ~~anions~~ compared to kosmotropic anions. An atom-level description of the structural dynamics of weak and strong polyelectrolyte brushes in response to variation in environmental stimuli can provide detailed information on the molecular interactions underlying the process.

Therefore, we have investigated the conformational dynamics of PMETAC and PDMAEMA brushes under different conditions of pH, solvent and salt types via atomistic MD simulations. PDMAEMA poly(dimethyl aminoethyl methacrylate) and PMETAC poly(2-(methacryloyloxy)ethyl[SEP]trimethylammonium chloride) have been ~~chosen~~ selected since these polymers are canonical models for weak and strong polyelectrolytes respectively. We have assessed the performance of the ~~chosen~~ selected atomic parameters to reproduce experimental swelling coefficients derived from ellipsometry measurements for brushes from 5 to 15 nm thickness. These atomistic simulations capture qualitatively the experimental trends for varying protonation states of PDAMEMA, for PDMAEMA and PMETAC in good and bad solvents and

for PMETAC in aqueous solutions of sodium chloride, sodium sulfate and sodium perchlorate. The models provide an atom-based description of the conformational dynamics and molecular interactions governing brush swelling/collapsing under different environmental conditions. In addition, the modular nature of the molecular models allows for the easy extension of these atomic parameters to a large variety of polymers or copolymers.

METHODS

Materials. 2-(Dimethylamino)ethyl methacrylate (DMAEMA), iodomethane, copper chloride (Cu(I)Cl), copper bromide (Cu(II)Br), 2, 2'-bipyridyl (bipy), anhydrous toluene and triethylamine were purchased from Sigma-Aldrich and used as received. 3-Trimethoxysilylpropyl 2-bromo-2methylpropionate (silane initiator) was from Fluoro Chem and stored at -20°C. Cu(I)Cl was kept in a desiccator, under vacuum, until used. All chemicals and solvents were analytical grades unless otherwise stated. Silicon wafers (100 mm diameter, <100> orientation, polished on one side/reverse etched) were purchased from Compart Technology Ltd and cleaned with Plasma System Zepto from Diener Electronic for 10 min in air plasma before use.

Deposition of ATRP silane initiator on silicon wafers. Following protocols adapted from the literature,{Husseman, 1999 #848} a piece of plasma-oxidized silicon wafer was immersed in a solution of silane initiator (30 μL) and triethylamine (50 μL) in anhydrous toluene (30 mL), and left at room temperature overnight. Then the wafer was rinsed with ethanol and dried in a nitrogen stream. Initiator-coated wafers were kept under nitrogen in dry condition until needed. The dry thickness of initiator layers was 2 nm, as measured by ellipsometry (JA Woollam spectroscopic ellipsometre).

Surface-initiated ATRP of PDMAEMA brush on silicon wafers. Poly(dimethylaminotethyl methacrylate) (PDMAEMA) brushes were synthesised from the initiator moieties via a 'grafting from' method, using atom transfer radical polymerisation (ATRP), following protocols adapted from the literature.{Sanjuan, 2007 #770;Tan, 2011 #822;Ayres, 2007 #849} To study PDMAEMA brush growth and the evolution of its thickness as a function of time, a solution of Cu(II)Br (18 mg, 80 μmol), bipy (320 mg, 2.05 mmol), and DMAEMA (42 mmol, 6.6 g) in water/ethanol (4/1 (v/v), 30 mL) was degassed using argon bubbling for 30 mins. Cu(I)Cl (82 mg, 828 μmol) was added to this solution and the resulting mixture was further degassed for 30 min before transferring 2 mL monomer solution to reaction vessels which contains silane initiator-coated silicon wafer (1 cm x 1 cm) under inert atmosphere. The polymerisation was stopped at different time points by immersing the coated substrates in deionised water, followed by washing with copious amounts of ethanol and drying in a nitrogen stream. The dry thickness of PDMAEMA brush was measured with ellipsometry. Initiator-deposited silicon wafers were cut into 1 cm x 3 cm for characterization via *in situ* ellipsometry. 5 nm and 10 nm of PDMAEMA brushes were prepared by stopping the polymerisation after 1.5 and 3 min, respectively according to PDMAEMA brush growth kinetics profiles.

Synthesis of poly(poly((2-(methacryloyloxy)ethyl)-trimethylammonium chloride) PMETAC brushes via quaternization of PDMAEMA brushes

Quaternisation of PDMAEMA brushes was carried out by immersing PDMAEMA brush (5 and 10 nm) coated silicon wafers in 1 % iodomethane DMF solutions overnight at room temperature, adapting protocols developed in the literature.{Sanjuan, 2007 #770} The wafers were then washed with ethanol and dried in a nitrogen stream. Dry PMETAC brush thicknesses were measured by ellipsometry. The final thickness of PMETAC brushes functionalized from 5 nm PDMAEMA

brushes was 7.5 nm, and 15 nm for those generated from 10 nm PDMAEMA brushes, in agreement with the expected increase in molecular weight and volume of the repeat units.

***In situ* ellipsometry.** *Effect of different pH and solvents.* The swelling of PDMAEMA and PMETAC brushes coated on silicon wafers was characterized in different conditions via in situ ellipsometery (using a flow chamber with a working volume of 1 mL). An argon flow was applied to dry the brushes in the chamber for 10 min prior to recording dry brush thicknesses. For PDMAEMA brushes, 4 mL of pH 5 aqueous solution were injected in the chamber and the system was allowed to equilibrate for 5 min before measuring the corresponding swollen brush thickness. Then, 4 mL of pH 9 aqueous solution were injected and the swollen brush thickness was measured after 5 min of equilibration. For PMETAC brushes, after drying with argon, 4 mL of anhydrous cyclohexane was injected and the brush thickness was recorded. Then the chamber was dried with argon before injecting 4 mL of deionised water and measuring the brush swollen thickness.

Effect of different salt type and concentration. To study the effect of salt type and concentration on the swelling of PMETAC brushes, sodium chloride, sodium sulfate and sodium perchlorate aqueous solutions at concentrations ranging from 10 to 500 mM were flushed in the flow cell of our *in situ* ellipsometer. Brush thicknesses in salt solutions were measured 5 min after equilibration, from lowest to highest concentrations.

Computational Details. Atomistic molecular dynamics (MD) simulations were performed for polymers poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA) and poly[2-(methacryloyloxy)ethyl] trimethylammonium chloride (PMETAC) chemically tethered at one end to thiol initiators spatially fixed to represent the substrate surface (Figure 1). Simulated systems were composed of either 9 or 16 polymer chains with 96 monomers per chain. Each polymer chain

was covalently attached to a thiol initiator where the carbon atoms were positional-constrained. The explicit representation of the thiol initiators enables to account for interactions between polymer chains and the attachment surface. The space between each chain is such that polymer surface density is either 0.1 chains per nm^2 whereas the initiators were distributed at about 4 chains per nanometer square. The PDMAEMA block contains tertiary amine groups whose pKa ranges between 7.4 and 7.8 depending on molecular weight.{van de Wetering, 1998 #679} Therefore, molecular models were built to represent the protonated (charged) and deprotonated (neutral) states of PDMAEMA. The effect of pH on PDMAEMA brushes was emulated through the assignment of protonation ratios associated to a given pH. The protonation/deprotonation sites were chosen randomly. Different monomer clusters were protonated/deprotonated during a single run while maintaining the protonation ratio constant. MD simulation of PDMAEMA in presence of 20 mM of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was also performed. All simulations were performed in explicit solvent (Table 1). Atomic coordinates for the initial conformations of the polymers and initiators were obtained through in-house built modeling tools followed by distance geometry optimization, and energy minimization. The GROMOS parameter set 54a7{Schmid, 2011 #774} was used to represent the polymers, the thiol initiators and the cyclohexane molecules, while the 53a6 parameter set was used to describe the ions.{Oostenbrink, 2005 #711;Oostenbrink, 2004 #686} The HEPES topology and atomic parameters were taken from the Atomic Topology Builder database.{Malde, 2011 #857} This topology has been modified at the piperazin group to represent the protonated state of HEPES at neutral pH. Accordingly, new atomic charges have been assigned to the piperazin charge group. The SPC water model was used in all simulations.{Berendsen, 1987 #827} Atomic charges for polymer monomers and HEPES molecule were calculated with the Hartree-Fock Self-Consistent Field theory and the 6-31G* basis

as described in RESP methodology.{Bayly, 1993 #688} The topologies and atomic parameters used in the simulations are available in the supplementary information (Tables S1 and S2: atomic charges, dihedral torsional potentials, potentials for bond stretching, bond-angle bending and improper dihedral deformation). Atomic parameters, topologies and coordinates of the polymer chains for PDMAEMA (neutral and charged) and PDMETAC attached to thiol initiators in water and cyclohexane can be found on the supplementary information or downloaded from dqfnet.ufpe.br/biomat or upon request.

Energy minimization was performed for all the systems until a mean force of lesser than 10 $kJ.mol^{-1}.nm^{-1}$ was achieved. MD simulations were performed in the NPT ensemble with a time step of 2 fs under periodic boundary conditions (PBC). Initial velocities were taken from a Maxwell distribution at 300 K and 1 atm. Bond lengths within the solute and the geometry of water molecules were constrained using the LINCS algorithm.{Hess, 1997 #681} The temperatures of solute and solvent were controlled by separately coupling them to a velocity rescaling thermostat with a relaxation time of 0.4 ps.{Bussi, 2007 #858} The pressure was maintained at 1 atm through the Berendsen pressure coupling algorithm with a coupling constant of 1 ps and an isothermal compressibility of 4.5×10^{-5} $(bar)^{-1}$ as appropriate for water and $1.137x\ 10^{-4}$ $(bar)^{-1}$ as appropriate for cyclohexane.{Berendsen, 1984 #875;Aicart, 1982 #842} Semi-isotropic coordinate scaling coupling was applied to all simulations. Long-range electrostatic interactions were calculated using the reaction field approach{Tironi, 1995 #828} with a relative dielectric permittivity constant of 66 for systems in water{Essex, 1998 #837;Glattli, 2002 #838} and 2.012 for the ones with cyclohexane.{Szklarczyk, 2015 #839;Papanastasiou, 1991 #841} The long-range electrostatic correction was applied to the interactions beyond a cutoff of 1.4 nm. Atom pair-lists were updated at every 5 fs. MD simulations were run for 200 ns with all systems reaching full

convergence of swelling around 75-80 ns (Figure S1). The MD simulation for HEPES in PDMAEMA was run for 400 ns. All simulations were performed using the GROMACS 4.6.5 suite of programs.{Hess, 2008 #682} Quantum chemistry calculations were performed with the NWChem software.{Valiev, 2010 #687} Analyses of the trajectories were performed with GROMACS 4.6.5 and in-house built codes. The simulated systems were oriented within the PBC box so that brush chains were parallel to the z-axis and perpendicular to xy-plane.

RESULTS AND DISCUSSION

We have validated the atomic parameters used in the brush simulations with respect to their suitability to describe three properties, pH, solvent quality and ion specificity, against experimental data obtained on identically synthesized polymer brushes. The experimental data presented here aimed to be directly compared to the atomistic model calculations. Polymer brushes were modeled as covalently attached to initiators in explicit solvent under periodic boundary conditions with semi-isotropic pressure scaling. In order to limit the length of calculation times and enable adequate MD sampling time, we aimed for polymer brushes in the range of 5 to 10 nm (dry thicknesses), *i.e.* 90 repeat units per polymer chain. We observed conformational changes in good agreement with the literature.

pH-Responsive swelling behavior of PDMAEMA brushes. PDMAEMA brushes have a pKa value near 7.5, *i.e*. they are protonated (charged) under low pH conditions and deprotonated (neutral) at higher pH,{van de Wetering, 1998 #679} leading to different conformational regimes. Although this behavior is well documented, we confirmed its occurrence in the present study via *in situ* ellipsometry (Figure 2). Well-defined brushes of thicknesses comparable to those of the computational models were grown, *i.e.* 5 and 10 nm, from silicon wafers via ATRP. As expected,

we observed relatively large swelling coefficients of 3.0 ± 0.2 and 2.9 ± 0.1 for 5 and 10 nm dry brushes, respectively, in pH 5 aqueous solutions, whereas exposure to pH 9 aqueous solutions, these brushes collapsed to heights of 6.9 ± 0.6 and 13.1 ± 1.7 nm. This is in good agreement with swelling ratios observed in the literature and the expected electrostatic repulsion between repeat units and adjacent chains upon protonation. Overall, PDMAEMA brushes displayed the typical swelling observed at low pH with swelling factor 3.0 and 2.9 for 5 and 10 nm brushes, respectively, whereas they collapsed to almost dry levels at higher pH with swelling factor 1.19 and 1.26 for 5 and 10 nm, respectively. The available literature reports PDMAEMA swelling coefficients in the range of 1.4-3.5 and 2.5-5.0 above and below the pKa of tertiary amine groups, respectively. [13, {Moglianetti, 2010 #817;Sanjuan, 2007 #770;Zhang, 2017 #758}] Our measurements are in in good agreement with these values,{Krishnamoorthy, 2017 #700;Sanjuan, 2007 #770;Zhang, 2017 #758} although slightly lower than values obtained from neutron reflectivity experiments.{Moglianetti, 2010 #817} Differences in the swelling ratios reported may be assigned to differences in the substrates supporting the brush, grafting densities achieved, polydispersities, polymer brush dry thickness, homogeneity and the technique used for their measurement.

We also simulated different protonation states of PDMAEMA brushes in order to characterize the polymer conformational dynamics at different pH conditions. ~~The pKa of the tertiary amine groups of PDMAEMA ranges from 7.4 to 7.8 depending on molecular weight.{van de Wetering, 1998 #679}~~ At high pH, the deprotonated PDMAEMA is expected to interact weakly with polar solvents such as water, as hydrophobic interactions between polymer chains prevail over hydration. Thus, a collapsed arrangement of the brush similar to dry state is expected and can be observed as a swelling coefficient close to 1 (Figure 3). On the other hand, at low pH, the protonated polymer favors electrostatic interactions with water molecules and ions, resulting in the complete solvation

of polymer chains. MD simulations of PDMAEMA at several protonation ratios ~~followed a clear trend: as the polymer protonation ratio increased (simulating decreased solution pH), the brush became more swollen (Figure 3) and reached a swelling coefficient of 1.8 for fully protonated polymer brushes (Figure 3), *i.e.* a height increase of 80 %. This is lower than the experimental value obtained (close to 3.0). However, this may be a result of the experimental brushes having some degree of polydispersity where longer chains swell to a higher degree, while the simulated brushes are perfectly monodisperse.~~ ~~The atomistic models of PDMAEMA~~ reproduce qualitatively the experimental trend with increase of the swelling coefficients in response to increase in the protonation ratio of the polymer chains (Figure 3). ~~Swelling coefficients were calculated for simulated systems containing 16 polymer chains, each chain with 96 monomers, covalently attached to a thiol initiator in one end of the chain.~~ However, the swelling coefficients underestimate the experimental measurements, *i.e.* 1.8 versus 3.0, and 1.0 versus 1.9 for fully protonated or deprotonated chains, respectively. There are a few potential explanations for the observed discrepancy. It may result from the denser grafting (σ = 0.5 chains/nm^2) in the experimental assays compared to the simulated systems (σ = 0.1 chains/nm^2), the expected heterogeneity of the synthesized polymer lengths and/or the dependency of the number of protonation sites with the distance as demonstrated by continuum models. {Borisov, 1994 #873;Zhulina, 1995 #874;Zhulina, 2011 #872} The investigation of charge inhomogeneity effects on brush thickness via atomistic simulations will likely require longer polymer chains since we have not observed significant change in brush thickness upon random assignment of different protonation sites while maintaining the same number of protons.

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The interdependence between the polymer conformations, hydration regime and pH was further examined through the analysis of density profiles along the z-axis (Figure 4a, 4d) and projected

onto the brush surface (Figure 4b, 4e). Two hydration regimes can be observed as a function of the protonation state of the polymer. These hydration regimes prompt distinct structural arrangements of the polymer brush (Figure 4). At high pH, PDMAEMA chains aggregate and remain collapsed along the initiator surface in the initial conformation used in the simulation (Figures 4a-c). This behavior has been quantified through the analysis of time-dependent changes in the angle θ between polymer chains and the axis normal to the brush surface (z-axis) (Figure S2). At values of θ = 0° the polymer chains are fully aligned with the z-axis in an extended conformation whereas at values of θ = 90° the chains are parallel to the initiator surface in a collapsed conformation. At high pH, water molecules do not diffuse into the hydrophobic core of the brush, and are localized exclusively on the brush surface (Figure 4a-4c). Conversely, at low pH, charged PDMAEMA is highly hydrated (Figure 4d-4f), and the average value of the angle θ decreases due to brush swelling (Figure S2). This is further shown by the full dispersion of the brush density along the vector normal to the substrate (Figure 4d) and the increase in solvent density between brush chains (Figure 4e). Hence, pH variation led to changes in the pattern of hydration of PDMAEMA chains consistent with changes in the protonation of the polymer repeat units and polymer conformational transitions expressed as brush swelling or collapsing (Figure 4c, 4f).

Solvent responsive behavior of PMETAC and PDMAEMA brushes. We next investigated the effect of good and bad solvents on the swelling behavior of polymer brushes (Figure 5). *In situ* ellipsometry experiments demonstrated the very clear solvent response of charged PMETAC brushes. In order to prevent hydration of PMETAC in air, we carried out dry measurements under an Argon flow. Similar hydration behavior, simply in response to acidic vapors, has been observed with polybase brushes previously.{Fielding, 2011 #856} In deionized water, PMETAC brushes

displayed relatively high swelling coefficients (2.2 ± 0.1 for 7.5 nm and 2.3 ± 0.2 for 15 nm brushes), in good agreement with the positive charge of their repeat units and associated electrostatic repulsion, leading to stretching of polymer chains upon chain hydration. In contrast, in cyclohexane, PMETAC brushes collapsed to comparable thicknesses observed in argon atmosphere.

The solvent responsive behavior of PMETAC was also captured in the MD simulations. We simulated the swelling behavior of neutral PDMAEMA and PMETAC brushes in different solvents (Figure 6), and assessed their conformational response to good and bad solvents via analysis of the corresponding density profiles (Figures 7 and 8). In polar solvents such as water, neutral PDMAEMA did not exhibit significant change in brush thickness (Figure 6) or brush chain hydration (Figure 7a-7c) from the initial collapsed conformation in the simulations. Likewise, in apolar cyclohexane neutral PDMAEMA remained mainly collapsed (Figure 6). However, cyclohexane molecules interacted with PDMAEMA polymer chains at the brush surface (Figure 7d-7f), leading to increase in chain disorder and in the calculated thickness (Figure 6). Similarly, in presence of cyclohexane, PMETAC also maintained a collapsed conformation with solvent molecules mostly restricted to the brush surface (Figure 8d-8f). Associated with this behavior, chloride counter-ions bind strongly to the positively charged ammonium groups of PMETAC throughout the simulations (Figure 8f). In contrast, PMETAC in water displayed a striking increase in brush thickness (Figure 6) due to hydration of the charged polymer chains (Figure 8a-8c). In water, chloride counter-ions were distributed within and outside the PMETAC brush, indicating full hydration (Figure 8f).

In summary, PMETAC brush conformations were strongly affected by the quality of the solvent. As reported in the literature,{Galvin, 2014 #767} we noted that brushes were partially swollen in

ambient air (data not shown), although the humidity level was not recorded. Therefore, in order to record true dry brush thicknesses, we flushed the *in situ* ellipsometric cell with argon (Figure 5). The thicknesses obtained were in good agreement with the expected increase in molecular weight of the repeat unit upon quaternization of PDMAEMA brushes with methyl iodide, based on changes in molar mass and volume of repeat units.{Tan, 2016 #852} In turn, whereas PMETAC brushes swell in aqueous solutions to comparable extended conformations observed for the corresponding PDMAEMA brushes, they remained collapsed in anhydrous cyclohexane. This is in good agreement with the swelling behavior typically reported for PMETAC brushes.{Sanjuan, 2007 #770;Zhang, 2017 #758} The swelling behavior of PMETAC in water and in cyclohexane has also been captured by the MD simulations (Figure 6). MD-derived swelling coefficients for PMETAC in water (good solvent) and cyclohexane (bad solvent) are 2.1 and 1.0, fairly comparable to the corresponding experimental values 2.2 and 0.8, respectively.

Ion-specific response of PMETAC brushes. In addition to solvent and pH effects, polymer brushes can respond to other stimuli such as changes in ionic strength and the chemistry of electrolytes present in the environment. Indeed, most polyelectrolyte brushes respond to changes in the ionic strength of the solution surrounding the polymer matrix, and often respond to the chemistry of electrolytes present in the medium.{Tan, 2011 #822} Such behavior is expressed as conformational transitions between extended and collapsed states. For this reason, properties such as brush thickness, mechanical properties, friction, and wettability can be modulated by changes in ionic strength and/or specific ion interactions, *i.e.* hydrophobic or ion-pairing collapse.{Kou, 2015 #819;Tan, 2011 #822;Willott, 2014 #818;Zhou, 2006 #850;Zhulina, 2014 #851} Therefore, we investigated the effect of sodium chloride (NaCl), sodium sulfate (Na_2SO_4) and sodium perchlorate ($NaClO_4$) on the swelling behavior of PMETAC brushes (7.5 nm dry thickness),

selected due to their well-reported responsive behavior in these conditions.{Tan, 2011 #822} Indeed, our experimental measurements confirmed that NaCl and Na_2SO_4 induce the swelling of PMETAC brushes (at concentrations ranging between 1 and 500 mM, see Figure 9). In both salt types, a gradual decrease in swelling was observed as the ionic strength increased, in agreement with the expected salting out behavior of polyelectrolytes.{Sanjuan, 2007 #770;Willott, 2014 #818} We noted however that although similar swelling trends were observed for thicker PMETAC brushes (30 nm dry thickness), no marked salting out was observed in the range of concentrations tested (Figure S3). In contrast, $NaClO_4$ (at a concentration as low as 1 mM) caused the complete collapse of PMETAC brushes (Figure 9). This is in good agreement with the chaotropic effect of this ion and its reported impact on PMETAC brush conformations.{Kou, 2015 #819} In summary, PMETAC brushes displayed a complex response to electrolytes. Thin brushes (7.5 nm dry thickness) displayed a gradual decrease in thickness in NaCl and Na_2SO_4 solutions (Figure 9a). However the decrease in swollen thickness was more pronounced for NaCl, as expected from the reduced kosmotropic character of this electrolyte compared to Na_2SO_4.{Hackett, 2015 #820;Kou, 2015 #819} In contrast, perchlorates led to complete collapse of PMETAC brushes, even at concentrations as low as 1 mM. This is in good agreement with their strong chaotropic character and reported PMETAC behavior in the literature.{Azzaroni, 2006 #821;Azzaroni, 2005 #823;Tan, 2011 #822} Interestingly, the effect of ionic strength on the swelling of thicker PMETAC brushes was weaker (30 nm, Figure S3), perhaps indicating that only part of the brush (*e.g.* the upper brush compartment) directly responds to electrostatic shielding in our experiments.

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MD simulations of PMETAC brushes in aqueous solutions of 150 mM of sodium chloride (NaCl), sodium sulfate (Na_2SO_4) and sodium perchlorate ($NaClO_4$) brushes were performed and

compared to the experimental data. As for all other simulations, PMETAC polymers were initially modeled in a collapsed configuration to emulate the brush in dry conditions (Figures 6 and 9). Addition of NaCl and Na_2SO_4 induced a conformational transition of the brush chains from collapsed to the extended state (Figure 9). This structural change led to a significant increase in brush thickness (Figure 9), which was associated to the full hydration of polymer chains and anions (Figure 10a-10f). The kosmotropic NaCl and Na_2SO_4 anions surrounded by PMETAC polymer chains are fully hydrated as shown by the homogeneous distribution of water molecules and ions within the brush (Figure 10a-10f). Conversely, $NaClO_4$ preserved the collapsed conformation of PMETAC chains, and brush thicknesses increased to a much lower extent (Figure 9). In the MD simulations, this behavior derives from strong interactions between the perchlorate ions and surrounding quaternary ammonium groups in PMETAC (Figure 10g-10i). We have examined this hypothesis through the analysis of radial distribution functions for ammonium groups in PMETAC, salt anions, and water oxygen (Figure S4). It further asserted the low hydration of PMETAC brushes in $NaClO_4$ solution compared to the highly hydrated PMETAC in NaCl and Na_2SO_4 (Figure S4a). Above all, the analysis reveals that $NaClO_4$ forms strong ion pair interactions with the tetramethylammonium group of the PMETAC while interacting weakly with water molecules (Figure S4b). The resulting ion-paired brush lacks any significant amount of entrapped water (Figure 10g-10i). These findings are consistent with the chaotropic nature of the ClO_4^- anion and with previous experimental measurements via atomic force microscopy and quartz crystal microbalance with dissipation (QCM-D).{Azzaroni, 2005 #823;Azzaroni, 2005 #823;Azzaroni, 2005 #823} On the other hand, Na_2SO_4 and NaCl interact strongly with the solvent (Figure S4c) but weakly with PMETAC ammonium groups (Figure S4b).

In brief, MD simulations of PMETAC exhibited an increase in swelling coefficients for NaCl and Na_2SO_4 and a decrease for $NaClO_4$ (Figure 9), consistent with the ellipsometry measurements. The kosmotropic chloride and sulfate interact strongly with water molecules, which form well-structured solvation layers around the ions (Figure S4c). In clear contrast, the chaotropic perchlorate interacts more strongly with the quaternary ammonium groups of PMETAC and less so with the solvent (Figure S4b-S4c). Consequently, hydrated NaCl and Na_2SO_4 display a homogenous distribution throughout PMETAC brush chains, inducing the brush swelling (Figure 10). On the other hand, $NaClO_4$ favors interactions with the PMETAC ammonium groups over the solvent molecules (Figure S4b-S4c), with lesser brush swelling compared to NaCl and Na_2SO_4 (Figure 10). The chosen atomic parameters reproduce qualitatively the salt-in and salt-out behavior of PMETAC induced by the NaCl and Na_2SO_4 versus $NaClO_4$ (Fig. 9). Yet, these parameters do not fully capture the subtle differences in the behavior of PMETAC in presence of the kosmotropic salts NaCl and Na_2SO_4 (Fig. 9). Na_2SO_4 induces a higher swelling of the brush compared to NaCl, while the opposite trend is observed in the computational simulations, albeit with a small difference between the two swelling ratios (Figure 9). We sought to address this inconsistence through the assessment of non-bonded interactions between the sulfate, water and ammonium group in PMETAC. The GROMOS force field describes van der Waals interactions through a Lennard-Jones 12-6 potential dependent on atom type. Accordingly, we assigned different atom types within the GROMOS parameter set 54A7 to the sulphur (S, Sdmso) and oxygen atoms (OM, OE, OA) in the sulphate group, and assessed how different combinations of atom types affected PMETAC swelling ratio. These parameters have not led to any significant improvement of the swelling behavior of PMETAC, and the use of atom types OM and S to represent SO_4^{-2} led to a swelling ratio similar to the values obtained for $NaClO_4$. Consistently, it was previously reported

that the combination of different Lennard-Jones parameters for SO_4^{-2} and water molecules did not improve the description of their association behavior using classical force fields.{Wernersson, 2010 #854} On the one hand, it has been shown that computational simulations must account for polarizability effects to better reproduce structural and thermodynamical properties of Na_2SO_4 in water[1].{Wernersson, 2010 #854;Jungwirth, 2003 #855} On the other hand, the inclusion of explicit polarization effects significantly increases computational costs with an apparently modest gain in accuracy. For instance, for a simple chemical system composed of Na_2SO_4 and water molecules, the calculated polarizability of 7 $Å^3$ considerably overestimates the experimental value of 4.418 $Å^3$.[70].{Li, 2017 #853} It is clear that more sophisticated models will be necessary to accurately differentiate PMETAC responsiveness to the two kosmotropic salts NaCl and Na_2SO_4. It is, however, necessary to balance the computational cost associated with the inclusion of explicit polarizability in our models and adequate sampling of temporal-spatial scales for brush dynamics.

Modeling the adsorption of HEPES on PDMAEMA brushes. We have investigated the interactions between HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), a routinely used organic buffer, and PDMAEMA brushes as first application of the developed atomic parameters. Previous work showed that HEPES reduces the binding affinity of plasmid DNA to PDMAEMA brushes, in turn reducing transfection efficiency.{Krishnamoorthy, 2017 #700} It was suggested that interactions between HEPES and PDMAEMA brushes might compete with DNA-brush interactions, therefore reducing the observed binding of plasmid DNA. We have assessed this hypothesis by performing MD simulations of PDMAEMA in 20 mM of HEPES at neutral pH where 50% of PDMAEMA repeat units are protonated. In these conditions, PDMAEMA brushes are well hydrated, exhibiting water pockets easily accessible from the surface (Figure 4d-4f). In the initial MD configuration, HEPES molecules were homogenously dissolved

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in the bulk solvent and rapidly diffused into water pockets within the brush (Movie SI-1). While in the bulk solvent phase, HEPES molecules formed transient dimers via electrostatic interactions between hydroxyl and/or sulphonic groups of neighboring molecules (Figures 11a-11b). As HEPES adsorbed onto the brush surface, these dimers persisted for longer lifetimes and aggregates of up to four molecules were formed deeper in the brush (Figures 11a-11b). HEPES molecules penetrate the brush via the sulphonic group, which interacts with the ammonium group in PDMAEMA (Figure 11c, Movie SI-1). Since HEPES remains hydrated upon penetration in the PDMAEMA brush at the simulated protonation state, the observed aggregation within the brush appears to be due to confinement rather than hydrophobic effect. These simulations suggest that HEPES may indeed compete with and hinder DNA diffusion into/interaction with PDMAEMA. Such interactions may therefore compete with DNA-brush interactions and result in a lower binding affinity. In addition, *in situ* ellipsometry had provided evidence for an opening of the conformation of PDMAEMA brushes when immersed in HEPES buffer. This observation is consistent with the present MD simulations, which indicate replacement of water molecules by HEPES and their aggregation upon confinement. Yet, the overall low HEPES density achieved in our system (all HEPES molecules diffuse to the brush and none are found in the bulk solvent phase) did not allow us to observe significant changes in brush conformation, and particularly in brush swelling. This contrasts with the very large excess of HEPES that is experimentally achieved in the macroscopic system. The modeling of interactions between oligonucleotides and cationic polymer brushes and their competitions with other small molecules such as HEPES are the focus of ongoing simulations where the introduction of a large excess of HEPES without depleting the bulk solvent phase will be dealt with via grand-canonical MD simulations.

DISCUSSION

The hydration of polyelectrolyte brushes has previously been studied experimentally in detail. Charged polyelectrolyte brush layers exhibit strong swelling in aqueous solutions as a result of hydration and electrostatic repulsion between brush repeat units, and collapse in apolar solvents with charged groups screened by ions from the aqueous solution.{Barbey, 2009 #735} In the case of weak polyelectrolytes such as PDMAEMA and PDEA, the hydration strongly depends on pH and the protonation states of repeat units.{Sanjuan, 2007 #770;Willott, 2014 #818} Hence, above the pKa of tertiary amine groups in PDMAEMA, brushes display swelling coefficients near 1.4-3.5, whereas at low pH, this coefficient is in the range of 2.5-5.0.{Moglianetti, 2010 #817;Sanjuan, 2007 #770;Zhang, 2017 #758} Differences in the swelling ratios reported may be assigned to differences in the substrates supporting the brush, grafting densities achieved, polydispersities, polymer brush dry thickness, homogeneity and the technique used for their measurement. This responsive behavior is also associated with a moderate sensitivity to the ionic strength at low pH, as positive charges in the side chains of repeat units are screened by free electrolytes, as evidenced by QCM-D and ellipsometry data.{Zhang, 2017 #758}

In comparison, strong polyelectrolytes do not typically respond to variations in pH but present complex conformational changes in response to variations in electrolyte type and ionic strength. For example, polycationic PMETAC brushes display different swelling behavior in presence of chaotropic anions, such as perchlorate ions, in comparison to more kosmotropic ions such as chlorides.{Alonso-García, 2013 #771} It was also shown that PMETAC brushes containing 67% water lost 17% water during collapse in 0.1 M NaCl, whereas the collapse in 0.1 M $NaClO_4$ led to a loss of 54% of entrapped water.{Alonso-García, 2013 #771} Similar observations were reported for other strong polycationic brushes such as poly (vinylbenzyltrimethylammonium chloride)

(PVBTMAC).{Bibi, 2012 #772} QCM-D data gave evidence for dramatic changes in brush hydration upon exposure to chaotropic ions. Indeed strong counter-ion condensation between the weakly hydrated trimethyl ammonium group and the weakly hydrated chaotropic ions such as SCN^-, ClO_4^- and NO_3^- ions resulted in the rapid collapse of the polymer brush. In contrast, weak ion pairing with kosmotropic anions such as $H_2PO_4^-$, F^-, HCO_3^- and Cl^- was observed. Similarly, Kou *et al.* studied the same Hofmeister anion series using PMETAC brushes and confirmed these observations.{Kou, 2015 #819} Indeed, they evidenced a stronger concentration-dependence of brush collapse in the presence of chaotropic anions compared to kosmotropic anions.

In order to validate the atomic parameters used in the brush simulations, we tested their prediction with respect to three properties: pH, solvent quality and ion specificity against experimental data obtained on identically synthesized polymer brushes. The experimental data presented here aimed to be directly compared to the atomistic model calculations. We observed conformational changes in good agreement with the literature. In order to limit the length of calculation times and enable adequate MD sampling time, we aimed for polymer brushes in the range of 5 to 10 nm (dry thicknesses), *i.e.* 90 repeat units per polymer chain. PDMAEMA brushes displayed the typical swelling observed at low pH (swelling factor 3.0 and 2.9 for 5 and 10 nm brushes, respectively, Figure 2), whereas they collapsed to almost dry levels at higher pH (swelling factor 1.19 and 1.26 for 5 and 10 nm, respectively). These values are in good agreement with the range of values reported in the literature,{Krishnamoorthy, 2017 #700;Sanjuan, 2007 #770;Zhang, 2017 #758} although slightly lower than values determined from neutron reflectivity experiments.{Moglianetti, 2010 #817} Likewise, the atomistic models of PDMAEMA reproduce qualitatively the experimental trend with increase of the swelling coefficients in response to increase in the protonation ratio of the polymer chains (Figure 3). Swelling coefficients were

calculated for simulated systems containing 16 polymer chains, each chain with 96 monomers, covalently attached to a thiol initiator in one end of the chain. However, the swelling coefficients underestimate the experimental measurements, *i.e.* 1.8 versus 3.0, and 1.0 versus 1.9 for fully protonated or deprotonated chain subunits, respectively. This discrepancy may result from the denser grafting ($\sigma = 0.5$ chains/nm^2) in the experimental assays compared to the simulated systems ($\sigma = 0.1$ chains/nm^2), the expected heterogeneity of the synthesized polymer chain lengths and/or a potential inhomogeneous distribution of protonation sites (REFS). In fact, implicit model simulations suggest a dependency of the number of protonation sites with the distance from surface.

PMETAC brush conformations were strongly affected by the quality of the solvent. As reported in the literature,{Galvin, 2014 #767} we noted that brushes were partially swollen in ambient air (data not shown), although the humidity level was not recorded. Therefore, in order to record true dry brush thicknesses, we flushed the *in situ* ellipsometric cell with argon (Figure 5). The thicknesses obtained were in good agreement with the expected increase in molecular weight of the repeat unit upon quaternization of PDMAEMA brushes with methyl iodide, based on changes in molar mass and volume of repeat units.{Tan, 2016 #852} In turn, whereas PMETAC brushes swell in aqueous solutions to comparable extended conformations observed for the corresponding PDMAEMA brushes, they remained collapsed in anhydrous cyclohexane. This is in good agreement with the swelling behavior typically reported for PMETAC brushes.{Sanjuan, 2007 #770;Zhang, 2017 #758} The swelling behavior of PMETAC in water and in cyclohexane has also been captured by the MD simulations (Figure 6). MD-derived swelling coefficients for PMETAC in water (good solvent) and cyclohexane (bad solvent) are 2.1 and 1.0, fairly comparable to the corresponding experimental values 2.2 and 0.8, respectively.

CONCLUSION

Solvent-polymer interactions are central to the behavior of polymer brushes. Furthermore, hydration of soluble ions, analytes, biomacromolecules and polymer chains play a large role in regulating their respective interactions.{Vogler, 1998 #773} Notably, the developed atomistic models have consistently reproduced the main features of the experimental measurements for pDMAEMA and pMETAC under different pH, solvents and salt type conditions. These models provide an atom-level portrait of the conformational dynamics of polymer brushes in response to a range of environmental parameters. Hence, these models not only capture chain conformation, but also provide a detailed picture of the localization of associated atoms. Moreover, polymer chain topologies were built so that monomer units are fully modular, which greatly simplifies the addition of novel monomeric units representative of a large variety of polymers or copolymers. Therefore the models presented should be applicable to the study of more complex molecular interactions between polymer brushes and analytes, as in the buffer-type specific response of PDMAEMA brushes or their strong capture of oligonucleotides, in particular RNA molecules.{Krishnamoorthy, 2017 #700;Li, 2018 #746}

Supporting Information. Atomic parameters used to build the polymer brush topologies; time evolution of brush thickness for PDAEMA and PMETAC simulations under different conditions of pH, solvent and salt types; orientation of the polymer chains in relation to the axis normal to the brush surface versus simulation time; *in situ* ellipsometric swelling coefficient for PMETAC brush in different types of salt aqueous solutions and concentrations; pair-correlation functions for quaternary ammonium groups in PMETAC, salt anions, and water oxygen atom; movie depicting the HEPES diffusion from bulk solvent into PDMAEMA brush generated from MD simulations.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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REFERENCES

TABLES

Table 1. Simulated Systems. Simulations were performed for DMAEMA and PMETAC brushes at different pH, solvents and salt types.

System	Solvent	Salts	Number of Atoms			Protonation State
			Anion	Polymer	Solvent	
PMETAC	CHX	Cl	1536	18432	39726	-
PMETAC	Water	Cl	1536	18432	158736	-
PMETAC	Water	ClO_4^-	7680	18432	158736	-
PMETAC	Water	SO_4^{2-}	3840	18432	158736	-
PDMAEMA	CHX	-	-	16896	47460	0 %
PDMAEMA	Water	-	-	16896	156354	0 %
PDMAEMA	Water	Cl	385	17281	184395	25 %
PDMAEMA	Water	Cl	767	17663	161397	50 %
PDMAEMA	Water	Cl	1152	18048	158202	75 %
PDMAEMA	Water	Cl	1536	18432	158736	100 %
[a]$PDMAEMA_{HEPES}$	Water	Cl	909	17663	53608	50 %

[a]This system contained 16 HEPES molecules and 115 mM NaCl in order to reproduce the concentration of major constituents of the HEPES buffer.

FIGURES

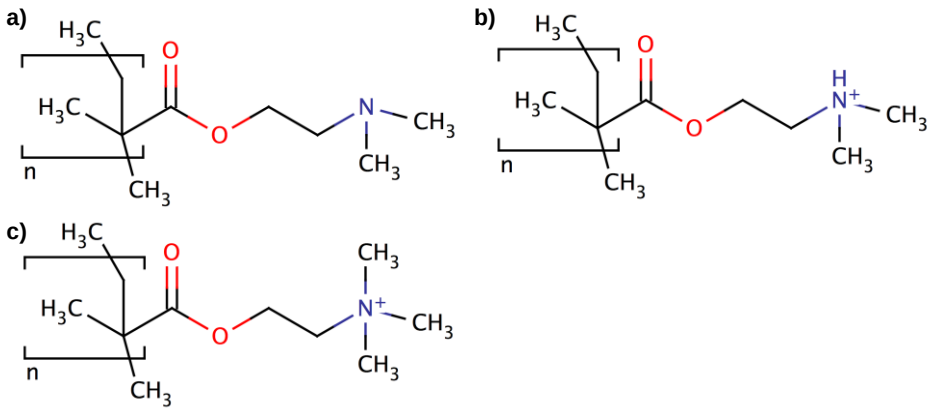

Figure 1. Chemical structures of the simulated polymer brushes. a) deprotonated PDMAEMA, b) protonated PDMAEMA and c) PMETAC.

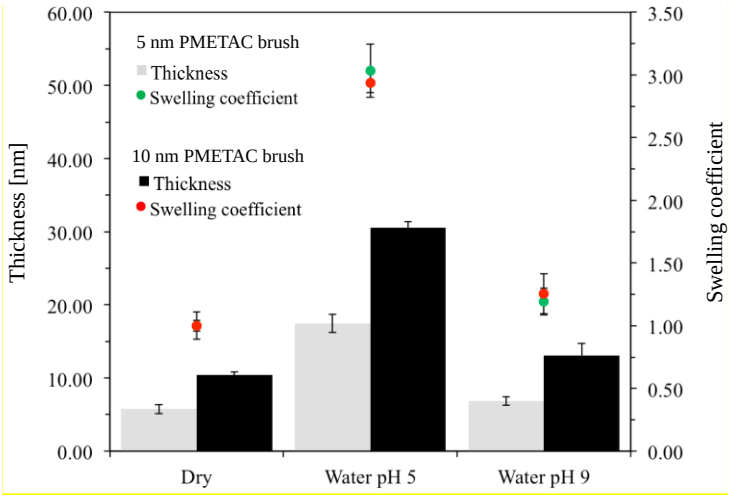

Figure 2. *In situ* ellipsometric thickness of 5 nm and 10 nm PDMAEMA brushes (dry brush thickness) in pH 5 (protonated brush) and pH 9 (deprotonated brush) aqueous solutions.

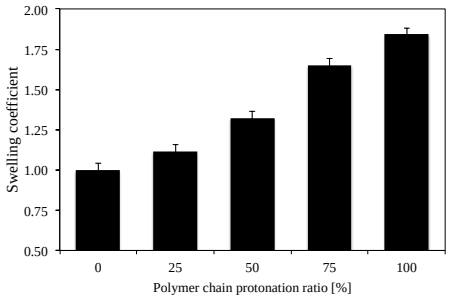

Figure 3. MD-derived swelling coefficients for PDMAEMA brushes as function of the degree of polymer chain protonation/deprotonation. The swelling coefficient is defined as the ratio between the maximal heights of the brushes in the wet and dry states (h_{max},wet/h_{max},dry).

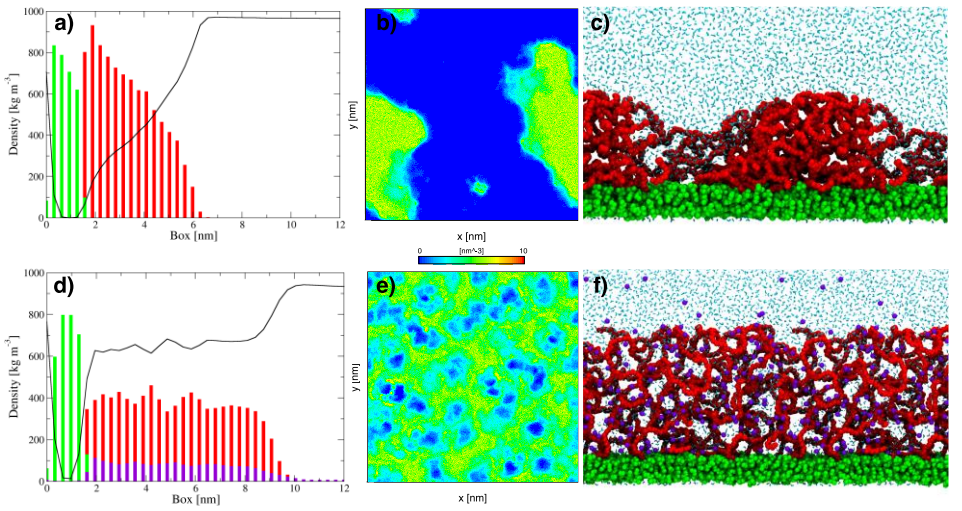

Figure 4. Swelling behavior of deprotonated and protonated PDMAEMA in water. Deprotonated (high pH) and protonated (low pH) PDMAEMA are shown in the top and bottom panels, respectively. a, d) Time-averaged density along the vector normal to the brush surface with initiators in green, brush in red, ions in violet and solvent in black. b, e) Time-averaged water density projected onto the brush surface (xy-plane). c, f) Final conformation obtained for protonated and deprotonated PDMAEMA in water with initiators shown in green, brush in red, ions in violent and solvent blue. Average calculated over the final 10 ns of simulation.

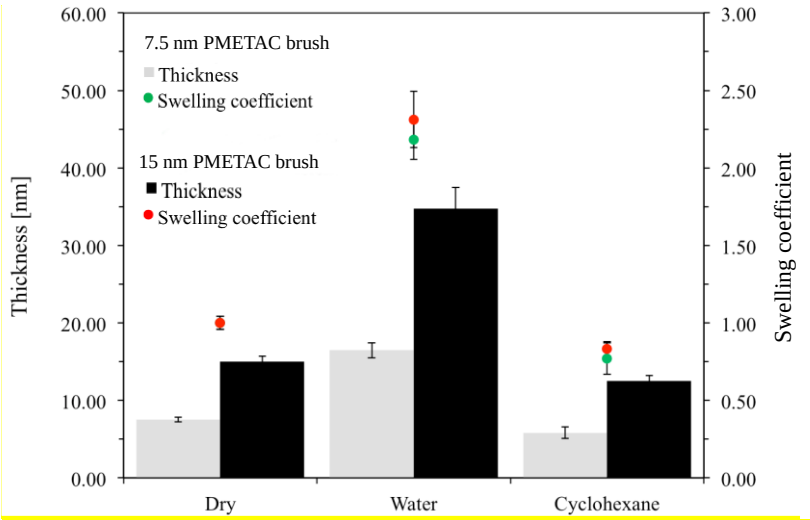

Figure 5. *In situ* ellipsometric thickness of 7.5 nm and 15 nm PMETAC brushes in argon atmosphere (dry brush thickness) and in good (water) and bad (cyclohexane) solvents.

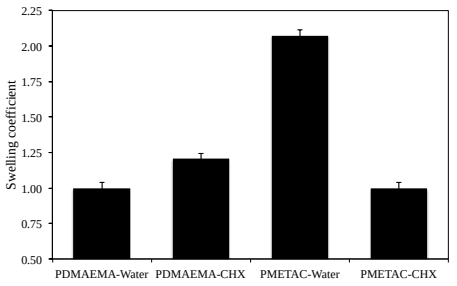

Figure 6. MD-derived swelling coefficients for PDMAEMA and PMETAC brushes in good and bad solvent. The swelling coefficient is defined as the ratio between the maximal heights of the brushes in the wet and dry states (h_{max},wet/h_{max},dry).

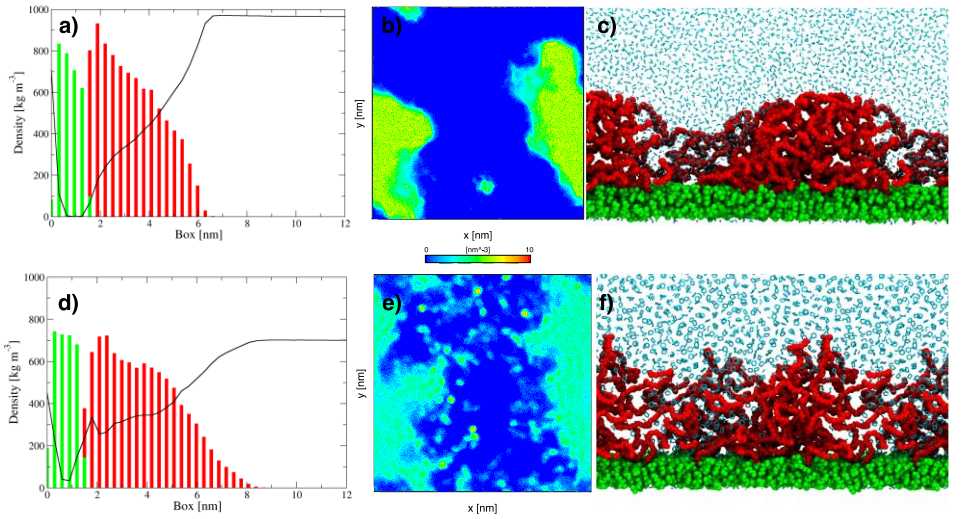

Figure 7. Swelling behavior of neutral PDMAEMA in water and in cyclohexane. PDMAEMA in water is shown in the top panel and in cyclohexane in the bottom panel. a, d) Time-averaged density along the vector normal to the brush surface with initiators in green, brush in red and solvent in black. b, e) Time-averaged water density projected onto the brush surface (xy-plane). c, f) Final conformation obtained for PDMAEMA in water and in cyclohexane, respectively, with initiators shown in green, brush in red, ions in violet and solvent blue. Averages over the final 10 ns of simulation.

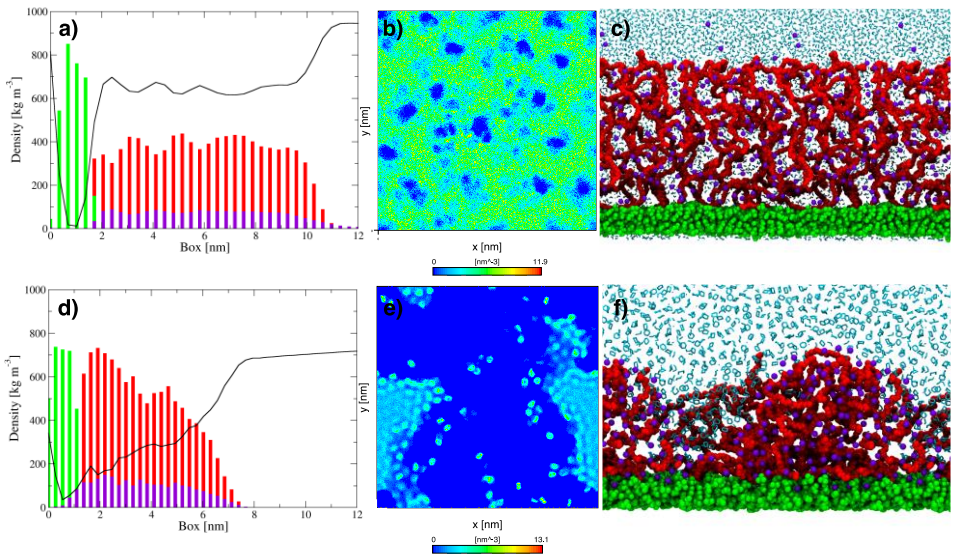

Figure 8. Swelling behavior of PMETAC in water and in cyclohexane. PMETAC in water is shown in the top panel and in cyclohexane in the bottom panel. a, d) Time-averaged density along the vector normal to the brush surface with initiators in green, brush in red, ions in violet and solvent in black. b, e) Time-averaged water density projected onto the brush surface (xy-plane). c, f) Final conformation obtained for PMETAC in water and in cyclohexane, respectively, with initiators shown in green, brush in red, ions in violent and solvent blue. Averages over the final 10 ns of simulation.

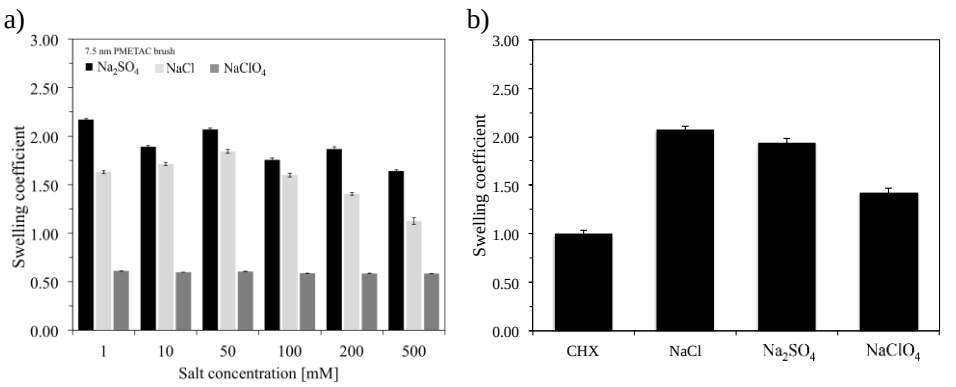

Figure 9. a) *In situ* ellipsometric swelling coefficient of 7.5 nm PMETAC brush in different types of salt aqueous solutions and concentrations. **b)** MD-derived swelling coefficients for PMETAC brushes in presence of sodium chloride, sodium perchlorate and sodium sulfate and in cyclohexane solvent. The latter is used as the simulation reference for dry PMETAC. The swelling coefficient is defined as the ratio between the maximal heights of the brushes in the wet and dry states (h_{max},wet/h_{max},dry).

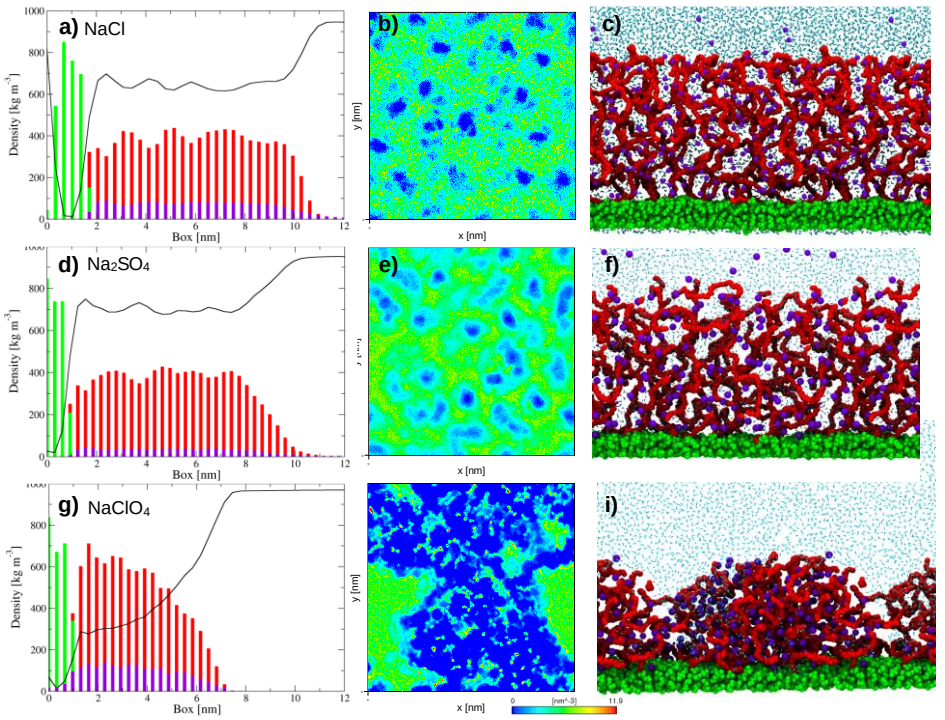

Figure 10. Swelling behavior of PMETAC in presence of different salt types. PMETAC in aqueous solution containing sodium chloride (top panel), sodium sulfate (middle panel) and sodium perchlorate (bottom panel). a, d, g) Time-averaged density along the vector normal to the brush surface with initiators in green, brush in red, ions in violet and solvent in black. b, e, h) Time-averaged water density projected onto the brush surface (xy-plane). c, f, g) Final conformation obtained for PMETAC in different salt solutions with initiators shown in green, brush in red, ions in violent and solvent blue. Averages over the final 10 ns of simulation.

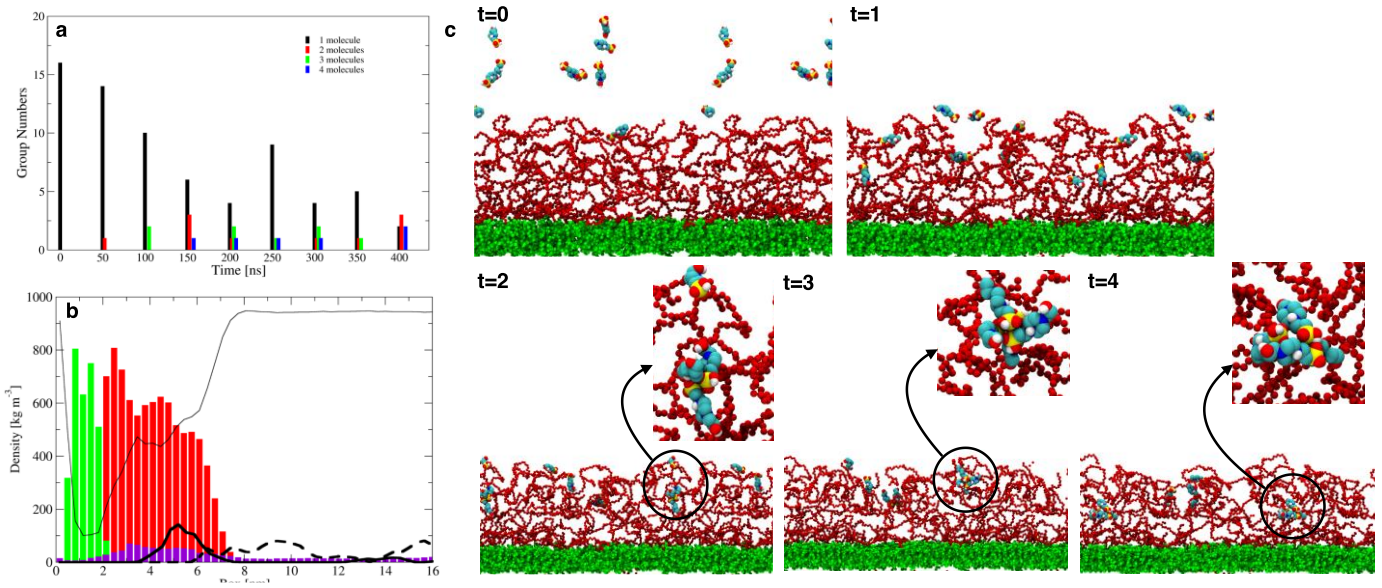

Figure 11. HEPES interaction with PDMAEMA (50% protonated) in water. a) Time-averaged density along the vector normal to the brush surface with initiators in green, brush in red, ions in violet and solvent in light black line, HEPES in the beginning and end of the simulation is shown in dark black and dashed lines, respectively. b) Averaged number of clusters formed by HEPES along the simulation time. c) Snapshots illustrating the cluster formation for a selected group of HEPES molecules. Averages calculated over the initial or final 10 ns of simulation.